PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of

Susumu WATANUKI, et al.

Docket No: Q92303

Appln. No.: 10/562,128

Group Art Unit: 1625

Confirmation No.: 5572

Examiner: David E. GALLIS

Filed: December 23, 2005

For: QUINOLONE DERIVATIVE OR SALT THEREOF

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Dr. Yuji Koga, hereby declare and state:

2. THAT I am a citizen of JAPAN;

3. THAT I have received the degree of Doctorate in Chemistry from the Graduate School of Science at the University of Tokyo, JAPAN;

4. THAT I have been employed by Yamanouchi Pharmaceutical Co., Ltd. (now Astellas Pharma Inc.) since April 1999, where I hold a position in Chemistry Research Laboratories, with responsibility for research and development of platelet aggregation inhibitors;

5. THAT I am familiar with the disclosure and claims of the above-identified patent application.

6. I am familiar with the prosecution of this application and I have reviewed the Office Action dated July 10, 2007, in the above-identified application.

7. In particular, I have reviewed the enablement rejection set forth in the Office Action, which is, that although the specification is enabling for a select compound of the elected subject matter, the specification is not enabling for all elected compounds. I disagree with the Examiner's conclusion, in view of the experimental data provided in the Declaration. The data provided in this Declaration demonstrate that several additional compounds of elected Group IV having different R^2 and R^{12} groups all possess both potent platelet aggregation inhibitory activity and potent P2Y12-ADP binding inhibitory activity.

8. I performed the following tests on the compounds shown in Table 1. In Table 1, the number shown in the "Ex Comp" indicates a number of Working Examples described in the specification of the present application.

(1) Test for Assaying Human Platelet Aggregation-Inhibiting Activity

9. Blood was withdrawn from healthy human volunteers (adult males), in the presence of a 1/10th volume of sodium citrate, and centrifuged-to obtain supernatant platelet-enriched plasma (PRP). The platelet count in PRP was determined with an automatic blood cell counter (MEK-6258; manufactured by NIHON KOHDEN CORPORATION). Then, the platelet count in PRP was adjusted to 3×10^8/ml with platelet-poor plasma. ADP as a platelet aggregation-inducing agent was a product of MCMEDICAL, Inc. Platelet aggregation was measured using an aggregometer (MCM Hematracer 212; MCMEDICAL, Inc.). Specifically, 80 μl of PRP and 10 μl of a solution of the compounds shown in Table 1 in a solvent (10% DMSO)

were incubated together at 37 °C. for one minute, to which 10 µl of ADP (50 µM) was added to induce platelet aggregation. Then, the change of the transmitting light was recorded over 5 minutes. Using the area-under-the curve of platelet aggregation as an indicator, the inhibition ratio was calculated. The results from the compounds of the invention at 10 µM (as final concentration) are shown in Table 1, in the "Test 1" column.

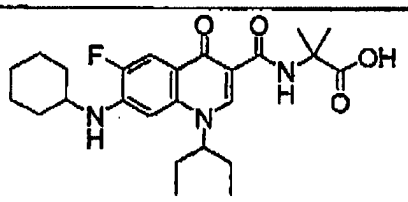
(2) Substitution Test of the Binding Between Human P2Y12 and 2-methylthio-ADP (2-MeS-ADP)

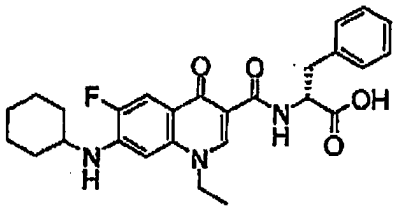
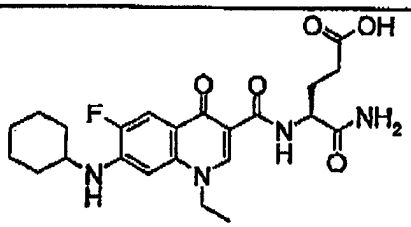
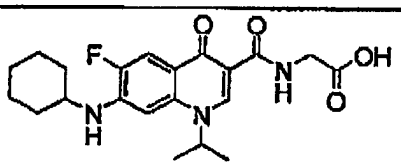
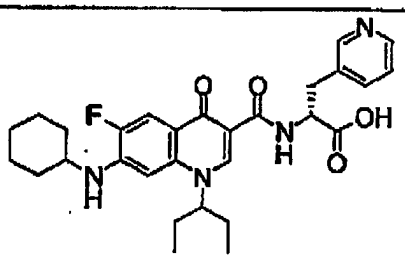
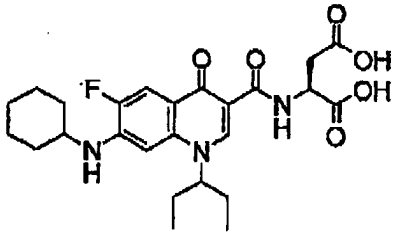
9. After C6-15 cells were inoculated on a DMEM culture medium to 1 x 10^6 cells in a 10-cm petri dish for culturing for one day, where 8 µg of a plasmid pEF-BOS-dhfr-human P2Y12 and 0.8 µg of pEF-BOS-neo (Nucleic Acid Res., 18,5322, 1990) were genetically introduced with a transfection reagent (LipofectAMINE 2000; manufactured by GIBCO BRL). 24 hours after the gene introduction procedure described above, the cells with the gene introduced therein were recovered and suspended in a DMEM culture medium containing 0.6 mg/ml G418 (manufactured by GIBCO BRL), to prepare serial dilutions; which were inoculated on a 10-cm petri dish. Colonies appearing 2 weeks later were individually obtained and defined as C6-15 cell expressing the protein P2Y12, for use in the following experiment (WO 02/36631, Mol. Pharmacol., 60, 432,2001).

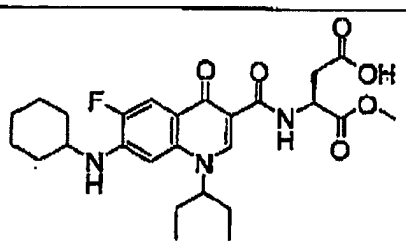
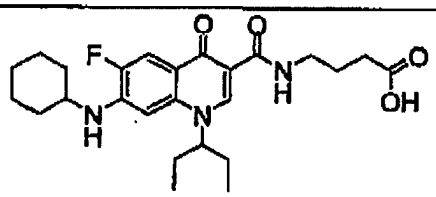
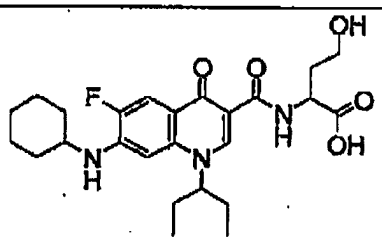
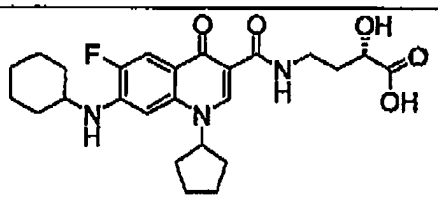
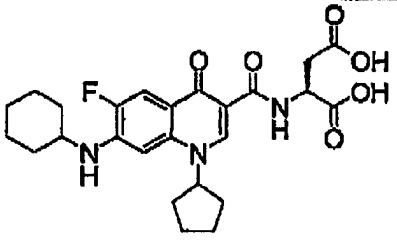
10. The C6-15 cell expressing the protein P2Y12 was cultured and recovered. After the cell was rinsed with PBS, the cell was suspended in 20 mM Tris-HCl, pH 7.4 containing 5 mmol/l EDTA and a protease inhibitor cocktail set Complete™ (manufactured by Boehringer Mannheim GmbH) and then homogenized with Polytron. After ultra-centrifugation, the resulting precipitate was suspended in 50 mM Tris-HCl, pH 7.4 containing 1 mM EDTA, 100 mM NaCl and Complete™. The resulting suspension was defined as a membrane fraction. 1.5 µl of the

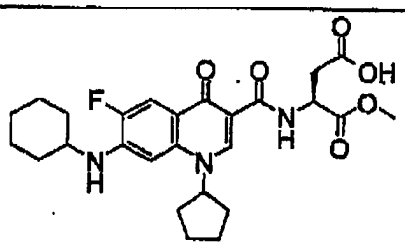
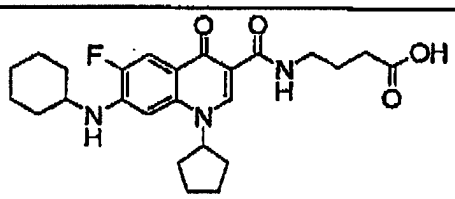
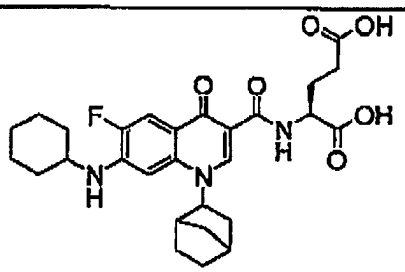
compounds of the invention, which are shown in Table 1 and 50 µl of 0.75 nM [^{3H}]-2-MeS-ADP (80 Ci/mmol; manufactured by Amersham Pharmacia Biotech) were added to 100 µl of the P2Y12 protein-expressing C6-15 cell membrane fraction (100 µg/ml) thus prepared, for incubation in 50 mM Tris-HCl, pH 7.4 containing 100 mM NaCl and 50 mM $MgCl_2$, at ambient temperature for one hour. Subsequently, the incubation mixture was recovered onto a glass filter with a cell harvester. Adding a microscintillator onto the glass filter, the radioactivity was assayed with a liquid scintillation counter. At the test, additionally, the radioactivity levels of such cell cultures with no addition of the compound and with addition of 1.5 µl of 100 µM 2-MeS-ADP were assayed as total binding and non-specific binding, respectively. Defining the total binding and the non-specific binding at inhibition ratios of 0% and 100%, respectively, the inhibition ratio (%) of an active ingredient or compound in accordance with the invention was calculated. The results from the compounds shown in Table 1 at 30 nM (final concentration) are shown in the "Test 2" column of the Table 1.

Table 1: Activity of Example compounds in platelet aggregation inhibition and ADP-P2Y12 binding inhibition

Ex. Comp.	Structure	Test 1 platelet aggregation inhibition (%inh.@10µM)	Test 2 P2Y12 binding inhibiton (%inh.@30nM)
	R^2=lower alkyl		
28		77*	63 (this data is shown in Table 2 of the specification as filed, at page 29)

172		70*	61
187		81*	13 (70@1μM)
255		73*	73
486		83	72
492		81*	68

515		77*	51
526		62*	45
534		77*	53
	R^2=Cycloalkyl		
406		91*	92
417		97	91

447		93*	86
467		73*	74
597	 (1RS,2RS,4SR)	90*	54

11. I hereby declare that, as can be seen from the data as set forth in Table 1 of this Declaration, Example compounds 28, 172, 187, 255, 486, 492, 515, 526 and 534 of the present application, which all contain a lower alkyl substituent at the R^2 position, exhibit significant inhibition of platelet aggregation when used at a concentration of 10μM. The Test 1 column of Table 1 displays percent inhibition of platelet aggregation in the presence of 10μM of the indicated Example compounds. All the above-mentioned compounds also show significant inhibition of ADP binding to P2Y12 at concentrations of 30nM; the Test 2 column displays percent inhibition of ADP binding to P2Y12. Notably, the above-mentioned compounds possess

different R^{12} groups, containing hydroxy, phenyl, pyridyl, $-CO_2R^0$ and $-C(O)N(R^0)_2$ substituents. Therefore, derivatives of the elected compound that have substituents other than $-CO_2H$ present at the R^{12} group exhibit both potent inhibition of platelet aggregation and potent inhibition of ADP binding to P2Y12.

12. I also hereby declare that compounds of Examples 406, 417, 447, 467 and 597 of the present application, which all contain a cycloalkyl group substituent at the R^2 position, exhibit significant inhibition of platelet aggregation when used at a concentration of 10μM. All these compounds also show significant inhibition of ADP binding to P2Y12 at concentrations of 30nM. As above, compounds 406, 417, 447, 467 and 597 also differ in the type of R^{12} group present.

13. I also hereby declare that for inhibition values indicated by "*" in Table 1 above, 10% DMSO-9% HPCD(hydroxypropyl-β-cyclodextrins) + 4.5% mannitol was added for assisting in solubilization of the compound, however, this change does not influence the outcome of the platelet aggregation and P2Y12-binding assays.

14. I also hereby declare that the data shown in Table 1 show that for compounds wherein X = C-R^7 (R^7=H), Y = C-R^6 (R^6=H), R^3 = a halogen, R^4 = a cyclohexyl, R^5 = H and R^{11} = H, the R^2 group can be a lower alkyl or a cyclohexyl substituent and exhibit both potent inhibition of platelet aggregation and potent inhibition of ADP-P2Y12 binding. Furthermore, as can be seen from Table 1, the R^{12} group can differ considerably in compounds wherein R^2 is either a lower alkyl or a cycloalkyl, without disrupting platelet aggregation inhibitory activity and ADP-P2Y12 binding inhibitory activity.

DECLARATION UNDER 37 C.F.R. § 1.132 Attorney Docket No.: Q92303
U.S. Application No.: 10/562,128

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: October 05, 2007

Yuji Koga
Dr. Yuji Koga